ARCHITECTURE OF THE OTOLITH END ORGAN: WITH SOME FUNCTIONAL CONSIDERATIONS

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ARCHITECTURE OF THE OTOLITH END ORGAN: WITH SOME FUNCTIONAL CONSIDERATIONS*

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SUMMARY PAGE

THE PROBLEM

The routine technique of temporal bone preparation usually includes the use of a strong fixative and decalcifier; therefore, the structural preservation of the fragile otolithic membrane in histological slides is uncertain. An attempt was made to preserve this structure as naturally as possible. The results obtained with three different decalcifiers are compared in a series of studies of squirrel monkey temporal bones.

FINDINGS

The best architectural preservation of the otolithic end organ was obtained after 10% formalin fixation, dehydration, celloidin embedding, and 10% EDTA decalcification.

The morphological features of this end organ are also discussed from the functional viewpoint. It is confirmed that, except for otoconia, basically both otolith and semicircular canal end organs have almost similar components.

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INTRODUCTION

Both the maculae of the otolith end organs and the cristae of the semicircular canals are mechanically stimulated in living individuals; therefore, it is important to know the relationship between the otolithic membrane, or the cupula, and the adjacent sensory epithelia in histological preparations. The otolithic membrane is, however, extremely fragile and will be destroyed easily by poor fixation, tonic change, inadequate temperature, strong chemicals, et cetera. The routine technique of temporal bone preparation for light microscopy usually includes the use of a strong fixative and a decalcifier from the acid group; therefore, the structural preservation of the otolithic membrane is usually uncertain in histological slides. Shrinkage or agglomeration of these structures is a most common appearance after using the routine procedure.

An attempt has been made to preserve, as naturally as possible, the architecture of the otolithic membrane and the adjacent structures in histological preparations. Three different decalcifiers were tried and the results compared in the present investigation.

MATERIALS AND METHODS

Thirty ears of the squirrel monkey (Saimiri sciureus) were used in the present study. All ears were from healthy, young, adult animals with no otological disease.

Except for the methods used in decalcification, all temporal bones were prepared in the same manner. They were fixed in 10 per cent formalin solution by intravital cardiac perfusion and/or by immersion, and decalcified. Only the EDTA group was decalcified after celloidin embedding. Following decalcification, all specimens were dehydrated in graduated percentages of ethanol (30, 50, 70, 80, 95, and 100 per cent) and ether-ethanol in a ratio of one to one. The specimens were embedded in 3 per cent celloidin for two weeks, in 6 per cent for three weeks, and in 12 per cent celloidin for three weeks. The extremely slow evaporation method was applied to harden the celloidin. All temporal bones were serially sectioned at 20 microns in the horizontal plane. To provide a pilot series, one of each ten sections was stained in hematoxylin-eosin, and examined by light microscopy.

For the purpose of determining the best method of preserving the otolithic membrane architecturally, the thirty ears were divided into three groups and prepared as follows.

Group 1

The temporal bones of seventeen ears comprise Group 1 which were processed without decalcification. After they had been embedded in celloidin and the celloidin had hardened, the blocks were immersed in a 10 per cent EDTA solution* for an average period of three weeks; shortest fourteen days, and longest thirty-five days. The solution was changed every other day (1, 4, 6-8, 10). Since no adequate chemical test was available to determine the end point of decalcification, a series of x-ray films (a minimum of four for each specimen) was taken for this purpose (5).

Group 2

Six ears constituted the group which, after fixation was completed, were decalcified by DECAL solution (Omega Chemical Corporation, New York), diluted three or five times with distilled water. Undiluted DECAL solution had been found previously to be too strong for inner ear structures. Because of trade secrecy, the exact ingredients of DECAL are not known; however, it is understood that the solution contains a diluted acid, various chelating agents, water softening agents, and others. The solution was changed once every other day, and the end point of decalcification was determined by a series of x-ray films. Thereafter, the specimens were dehydrated, embedded in celloidin, hardened, sectioned, stained, and microscopically examined.

Group 3

The routine temporal bone preparation technique was used in processing the seven ears of the third group. After fixation in 10 per cent formalin was completed, the temporal bones were decalcified in a solution of 5 per cent trichloroacetic acid which was changed once every two days. The end point of decalcification was determined chemically by using a 5 per cent ammonium oxalate and 5 per cent ammonium hydroxide mixture. Thereafter, the specimens were neutralized, dehydrated, embedded in celloidin, sectioned, stained, and examined.

RESULTS AND DISCUSSION

The best architectural preservation of the otolithic membrane was observed in the formalin fixed-EDTA decalcified inner ears (Group 1) (Figure 1). The otolithic zone was conspicuously thick in this group of ears, and crystal shaped otoconia was more frequently observed (Fibure 2). In trichloroacetic acid decalcified ears (Group 3), a rather thinner otolithic zone was observed, and otoconia itself appeared as hematoxylin dark-stained agglomerated granules (Figures 3,4). The findings of the otolithic zone in DECAL decalcified ears of Group 2 fell in between those of the other two groups (Figure 5).

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*Two different brands of EDTA were used. A 10 per cent solution was made either from 1) Sodium Tetra Ethylenediamine Tetraacetate Solution (concentrated-technical) (Fisher Laboratory Chemical), or 2) Disodium Ethylene Diamine Tetraacetate, Sequestrene Na_2, recrystalized (Geigy Industrial Chemicals).

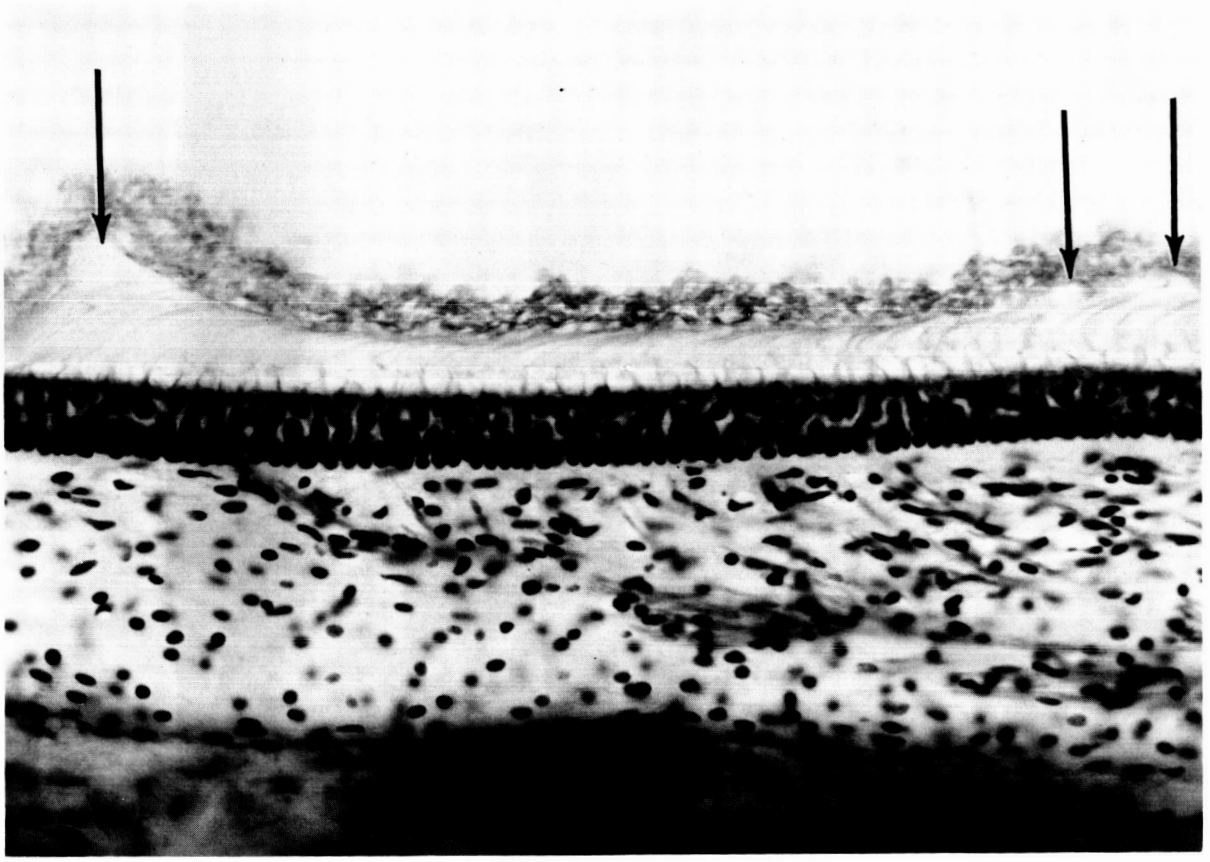

Figure 1

Macula Sacculi after EDTA Decalcification

Note spaces between otolithic zone and cupular zone (arrows). Horizontal section. 20 microns. Hematoxylin-eosin staining. 450 X

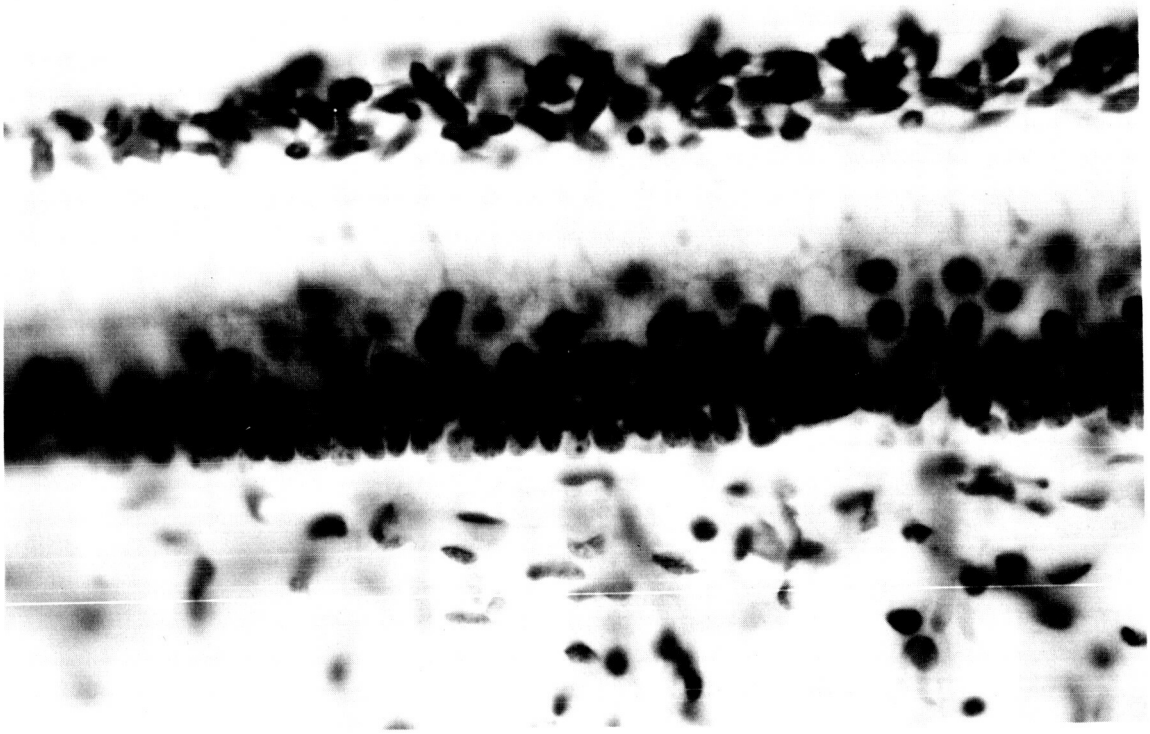

Figure 2

Large Crystal-Shaped Otoconiae on the Utricular Macula

EDTA decalcification. Horizontal section. 20 microns. Hematoxylin-eosin staining. 840 X

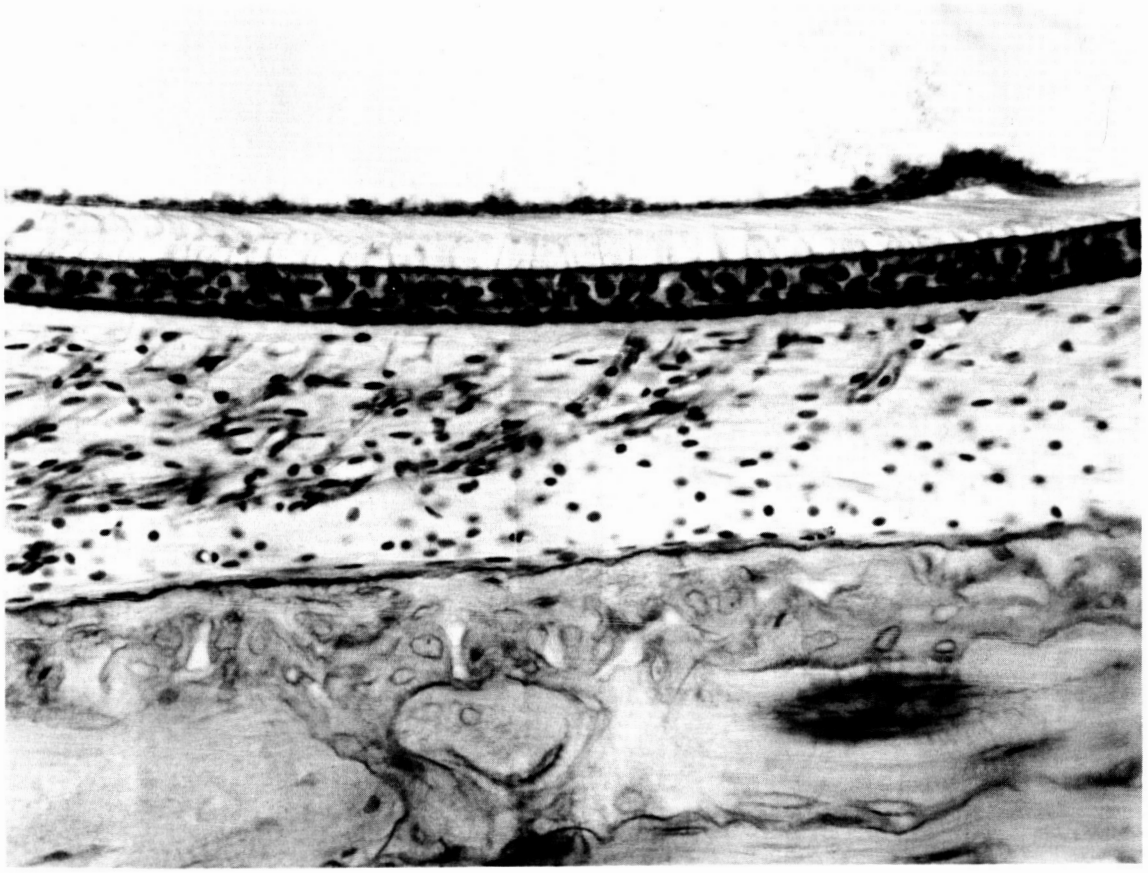

Figure 3

Macula Sacculi after 5 Per Cent Trichloroacetic Acid Decalcification

Note the flattening of the entire structure and thin otolithic zone. Horizontal section. 20 microns. Hematoxylin-eosin staining. 450 X

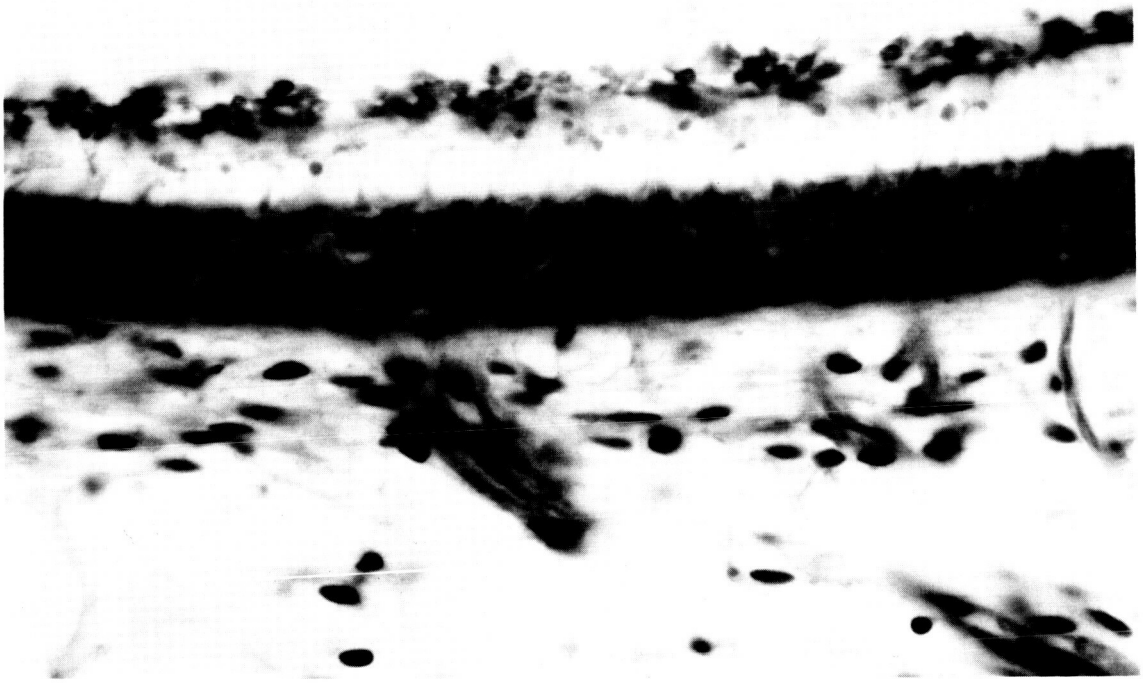

Figure 4

Agglomerated Otoconiae on the Utricular Macula after 5 Per Cent Trichloroacetic Acid Decalcification

Horizontal section. 20 microns. Hematoxylin-eosin staining. 840 X

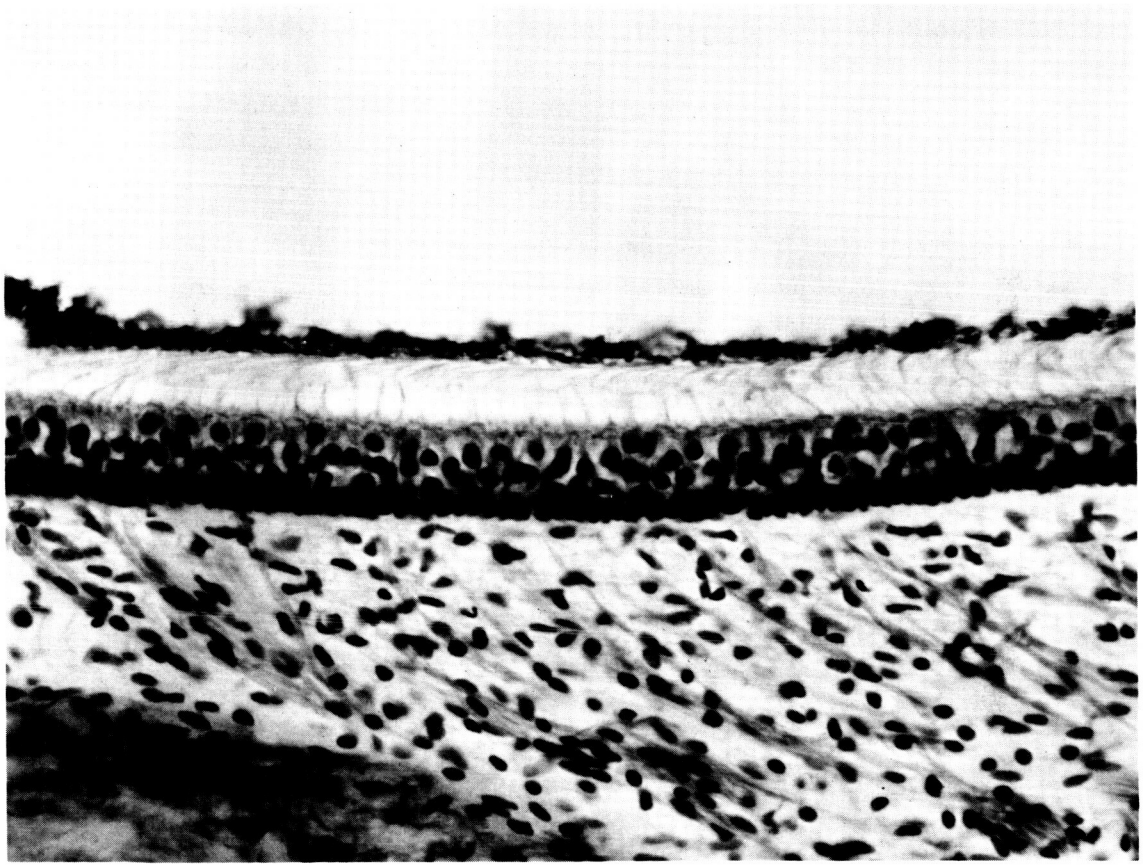

Figure 5

Macula Sacculi after Diluted DECAL Decalcification

Otolithic zone, cupular zone, and macula are not severely collapsed. Horizontal section. 20 microns. Hematoxylin-eosin staining. 450 X

With the use of the routine temporal bone preparation technique, which includes utilizing some acids, usually the zonal structure of the otolithic membrane can hardly be detected. To protect the tonic change of the otolithic membrane, Wittmaack (14) applied various chemicals through the round window of the guinea pig. No such attempt was made in the present investigation. Notwithstanding, three different zones of the otolithic membrane are very clearly distinguished after formalin fixation (perfusion and/or immersion), dehydration, celloidin embedding, and EDTA decalcification. Werner (11) had demonstrated the importance of warm perfusion solution at body temperature, without glacial acetic acid. In the present study, the formalin solution was used at room temperature without any acid, and the temporal bones in the fixative solution were kept exclusively in a refrigerator. Nevertheless, the structural preservation was excellent when no acid was used for the fixation or decalcification. The collapse of the structure is more likely caused by the use of acids.

The otolithic zone was clearly distinguishable from the cupular zone in the present study of the squirrel monkey ears. In other words, the otoliths are most probably located outside the cupular zone. The otolithic zone itself is almost flat, except for some wavy formations especially at the edge. At these wavy points, some spaces between the otolithic and cupular zones could be observed; therefore, it is unlikely that these two zones are directly connected (Figure 1). The content in these spaces is unknown.

The staining quality of the otoconia was good in both the DECAL decalcified group (Group 2) and the trichloroacetic acid decalcified group (Group 3), but was relatively poor in EDTA decalcified ears (Group 1). Investigation of the otoconia was extremely difficult in some of the EDTA decalcified ears, although they were stained darker than usual.

Should the condition of the otolith itself be under investigation, good control specimens prepared by the same preparation procedures (stated in detail) are definitely necessary. Otherwise, the pathological condition of the otolith cannot be discerned. Different cutting angle, intraspecial and intraindividual differences, et cetera, should also be considered. It is well known that not only the shape and size of the otolith (statoconia) but also the means of suspension of the statoconia are quite different among the different species (2,12,14).

The cupular zone appeared to be thick and was very clearly recognizable in the maculae of most of the EDTA decalcified ears. In most of the trichloroacetic acid decalcified ears, it usually appeared to be much thinner and sometimes could scarcely be seen.

According to Flock (3), the gelatinous cupula covers not only hair cells but also the mantle cells which are located at the edge of the lateral line organ. In that condition, the ciliae are totally enclosed in the cupula. In the present study of the squirrel monkey ears, the sensory ciliae always protruded into the subcupular zone, which is a definite open space between the cupular zone and the cuticular lamina of the hair cells. The content of this subcupular zone is unknown. In many instances the edge of the cupular zone of the squirrel monkey seemed to be attached to the epithelium which surrounds the sensory hair cells, seen especially in the ears of Group 1 and Group 2 of the present study. It is unlikely that this is the endolymph, inasmuch as this subcupular zone probably does not open freely to the endolymphatic space.

In the primitive form of this end organ, there is no specific zone between statoconia and the ciliae; in other words, the hairs of the hair cells are directly stimulated by the statoconia. The existence of the intermediate cupular zone may have an effect on the physical mechanism between the otolith and the sensory hair cells. The force from the otolith will be shared over a wider area, and may become less when it reaches the ciliae of hair cells. More hair cells can be stimulated simultaneously; therefore, the information from the well-developed otolith end organ might be much more complex than that from the primitive form.

In the present light-microscopic investigation, the points of the sensory ciliae reached to the cupular zone; however, it is still not known whether the ciliae are loosely attached to the cupular zone or partly embedded in it. It seems more likely to be the latter, as Smith (9) recently demonstrated that, in organ of Corti, the ciliae were partly embedded in the tectoria. Cupular substance is considered to be mucopolysaccharide because it reacts strongly to the periodic acid-Schiff reagent (13). The fragility of these structures makes it difficult to investigate these areas.

The sensory ciliae, hair cells, and supporting cells were well preserved in all of the present study groups, except for the thickness of the macula which appeared slightly thinner in the trichloroacetic acid decalcified ears, than in those of the other two groups (Figures 1,3,5).

From the studies of the horizontal serial sections, the shape of the macula appears to be more or less concave, and the direction of the sensory ciliae, especially at the periphery of the macula, is usually toward the center of the macula. The direction of the cupular filaments, however, is not necessarily the same. It looks more likely to be toward the one direction (Figures 1,5). The cupular filaments look amazingly similar both in the cupular zone and cupula (Figures 1,6).

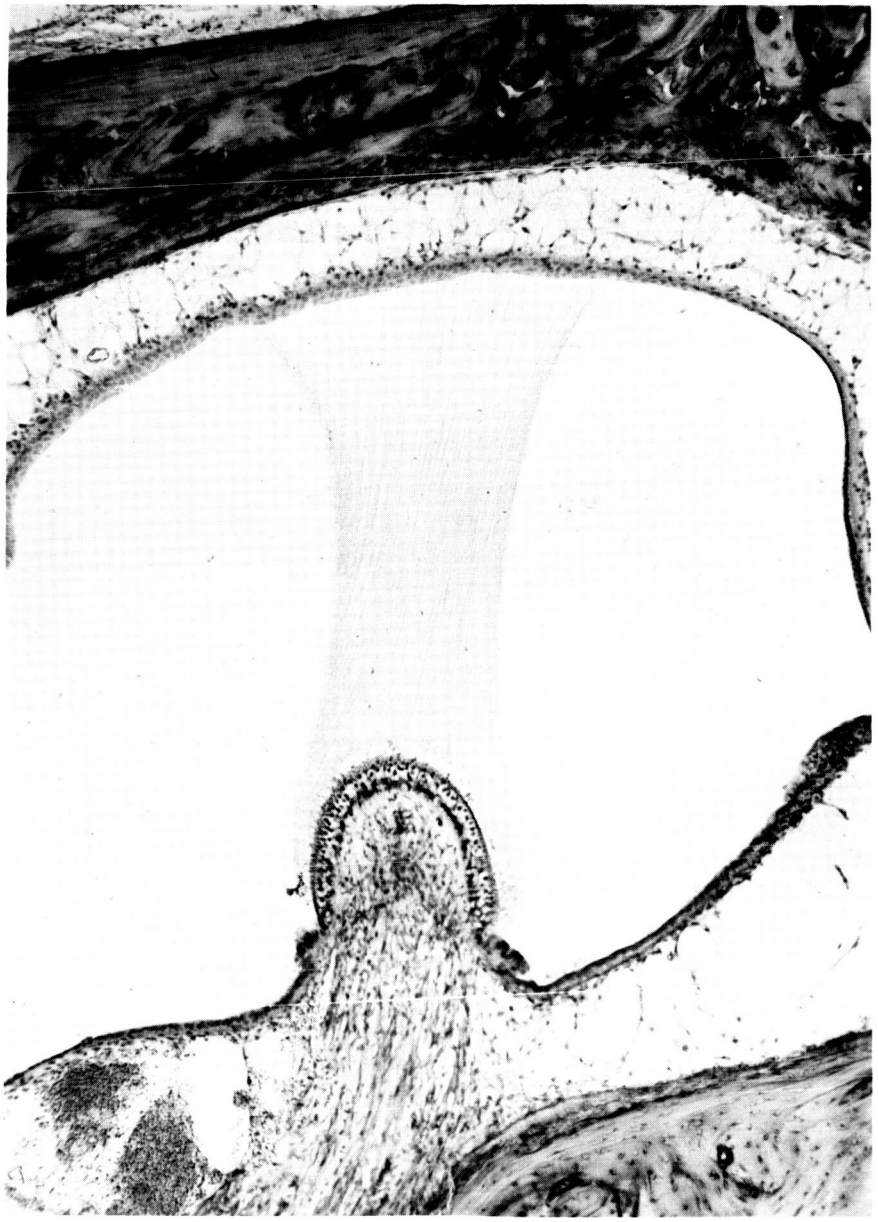

Figure 6

A View of Well-Preserved Cupula of the Lateral Semicircular Canal

Note the pattern of cupular filaments and that it extends to the opposite wall of the ampulla. Horizontal section. 20 microns. Hematoxylin-eosin staining. 70 X

Although the size, shape, and length of the cupula and cupular zone are quite different, the components of both the cupula-crista system and the otolithic membrane-macula system are amazingly similar, except for the existence of the otolith. A similar excitatory mechanism, therefore, can be expected at the level of ciliae-hair cells. The primary mechanism of the mechanophysical forces approaching these end organs looks quite different in otolith and in semicircular canal end organs; the former receives the force from the movement of the otolith and the latter, from the endolymphatic current. It is still possible, however, to consider that , to a certain extent, these two end organ systems might receive both angular and gravitoinertial forces, with definitely different thresholds.

The macula and otolithic membrane could appear to be slightly different in thickness, depending upon the different cutting plane and level; however, the difference is always minimal. In the present investigation, the attempt was made to cut all temporal bones in the same plane, and to compare structures at the same level. The differences in appearance of the otolithic membrane and macula among the three groups of the present study were quite obvious.

EDTA was found to be useful only at a pH of around 7.2 - 7.4. In a previous study, the desired decalcification could not be obtained, even after a considerably long period, when EDTA was used at an undesirable pH. The average periods needed for decalcification of a single temporal bone of the squirrel monkey were: 10 per cent EDTA, about 3 weeks; 20 per cent DECAL, 6-8 days; and 5 per cent trichloroacetic acid, 2-3 weeks. The only disadvantage of the first two methods is the necessity of a series of teleroentgenograms for detecting the end point of the decalcification.

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The otolithic membrane is extremely fragile and is easily destroyed by post-mortem changes, tonic change, strong chemicals, et cetera. The routine technique of temporal bone preparation usually includes the use of a strong fixative and decalcifier; therefore, the structural preservation of this structure in histological slides is uncertain. An attempt was made to preserve the otolithic architecture as naturally as possible. In studying squirrel monkey temporal bones the results obtained with three different decalcifiers are compared.

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